

Original Article

Mutagenicity and cytotoxicity in patients submitted to ionizing radiation A comparison between cone beam computed tomography and radiographs for orthodontic treatment

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ABSTRACT

Objectives: To evaluate and compare mutagenicity (micronucleus) and cytotoxicity (karyorrhexis, pyknosis, and karyolysis) in exfoliated buccal mucosa cells of children following cone beam computed tomography (CBCT) or conventional radiograph exposure necessary for orthodontic planning.

Materials and Methods: A total of 49 healthy children were submitted to CBCT or a conventional orthodontic radiographic protocol; they were divided into two groups based on exam: CBCT (n = 24) and Radiographic Set (n = 25) groups. The micronucleus test in the exfoliated buccal mucosa cells was applied.

Results: There was not a statistically significant difference ($P > .05$) found between the number of micronucleated buccal mucosa cells (MNC) before and after exposure to radiation in either group, showing that neither group experienced a mutagenic effect. However, radiation did cause other nuclear alterations closely related to cytotoxicity, including karyorrhexis, pyknosis, and karyolysis, in both groups ($P < .05$). The CBCT group presented a greater increase in cell death than was noted in the Radiographic Set group ($P < .044$).

Conclusion: According to the micronucleus test, mutagenicity was not induced by the CBCT or the conventional radiographs, but cytotoxicity was verified after these exams, especially after CBCT. That might have happened once the CBCT group received a greater radiation dose than the Radiographic Set group as a result of the protocols used in orthodontic planning for this study. (*Angle Orthod.* 2013;83:104–109.)

KEY WORDS: Micronucleus test; Buccal mucosa cells; Cone beam computed tomography; Dental radiography

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INTRODUCTION

The diagnostic radiation associated with orthodontic care has public health significance due to the high and increasing prevalence of orthodontic treatment, especially in young people.¹ This significance exists because ionizing radiation is able to cause single- and double-strand breaks and DNA-protein crosslinks.² When normal functioning of DNA repair genes and/or cell proliferation and differentiation control genes is lost as a consequence of mutations, the risk of cancer development increases.³

The genetic damage caused by genotoxic agents, such as ionizing radiation, can be measured using biomonitoring tests, and the micronucleus (MN) test is a very reliable assay for evaluating mutagenicity. This test is based on the formation of micronuclei from particles of chromatin material that, as a result of chromosome breakage or spindle dysfunction, do not migrate to the poles during anaphase and are not incorporated into the

telophase nuclei of the dividing cell and result in the formation of one or more small satellite nuclei in the cytoplasm of the daughter cells.⁴

The MN test utilizes a well-established protocol that is performed in human peripheral blood lymphocyte cultures. MN evaluations are made in buccal epithelial exfoliated cells (BEC) as well, and this test is considered to be the least invasive method available with which to measure DNA damage in humans.⁵ As a result of its ability to assess the activity of many chemical or physical carcinogenic and mutagenic agents in situ, the MN test in the BEC is the choice of many recent human biomonitoring studies, including those involving the following: alcohol,⁶ tobacco,⁷ oral cancer and other oral pathologies,⁸ and patients undergoing radiotherapy² or chemotherapy.⁹

With regard to ionizing radiation in dentistry, the MN test in the BEC was utilized to evaluate the panoramic dental radiograph,^{3,10–16} the lateral digital radiograph,¹⁷ mandibular cone beam computed tomography (CBCT),¹⁸ and orthodontic radiographs.^{19,20} In general, these studies revealed only cytotoxic effects,^{10–12,14–19} and only one study³ showed mutagenic results after exposure to the dental X-ray. None of the studies pointed out the effects of CBCT utilized for orthodontic planning in the BEC of children. In this investigation, the frequencies of the micronucleated cells (mutagenicity) in the buccal mucosa of individuals exposed to diagnostic methods used in orthodontic planning were compared using CBCT or a radiographic protocol. To monitor cytotoxic effects, karyorrhexis, pyknosis, and karyolysis were also evaluated in this setting. Finally, a comparison between the alterations caused by these exams was realized.

MATERIALS AND METHODS

Subjects

The study subjects included 49 healthy children. None were alcohol or tobacco consumers; they did not utilize mouth rinses or medicine; and they had not been submitted to ionizing radiation in the 16 days prior to the study. Patients were divided into two groups, as follows: (a) the CBCT group ($n = 24$; 14 males and 10 females; mean age 11 ± 1.2 years), all of whom partook in the following protocol: FOV 13 cm (120 kV, 46.72 mAs, 40 seconds) and FOV 22 cm (120 kV, 47.74 mAs, 40 seconds) were realized on the same day in each individual. All CBCT scans were taken at the same private establishment of dental radiology in Rio de Janeiro, RJ, Brazil, using the Classic i-CAT equipment (Imaging Sciences International, Hatfield, Pa). The CBCT FOV 22 cm was made to cover all of the structures necessary for orthodontic analysis, and CBCT FOV 13 cm was used to provide a

higher quality image of the dental structures. (b) the Radiographic Set group ($n = 25$; 15 males and 10 females; mean age 11.2 ± 1.4 years), who had the following radiographs taken: lateral cephalographic (LAT), posteroanterior (PA), panoramic (PAN), full periapical exam (six of the anterior teeth and eight of the posterior teeth), and bitewings (one on the left side and one on the right side). The radiographs were produced using the Rotograph Plus (Dabi Atlante, Ribeirão Preto-SP-Brazil; LAT: 80 kV/10 mA/1.3 seconds/0.003 mSv; PA: 85 kV/10 mA/1.6 seconds/0.03 mSv; PAN: 70 kV/10 mA/17 seconds/0.03 mSv) and Spectro 70X Seletronic (Dabi Atlante; anterior periapical: 70 kV/8 mA/0.4 seconds/0.008 mSv/round collimation; posterior periapical and bitewing: 70 kV/8 mA/0.45 seconds/0.008 mSv/round collimation). The Radiographic Set group data were taken from our previous study.²⁰

All exams were requested for orthodontic planning and treatment. The study was approved by the Institutional Human Ethics Committee (project No. 0071.0.239.000-09, approval No. 09/2010), and informed consent was obtained from the parents of the included individuals.

Micronucleus Test in Oral Mucosa Cells

Exfoliated buccal cells were collected immediately before X-ray exposure and after 10 days. After rinsing the mouth with tap water, cells were obtained by scraping the right/left cheek mucosa with a moist wooden spatula. Cells were transferred to a tube containing saline solution, fixed in 3:1 methanol/acetic acid, and dropped onto pre-cleaned slides. Later, the air-dried slides were stained using the Feulgen/Fast Green method and were examined under a light microscope at $400\times$ magnification to determine the frequency of micronucleated buccal mucosa cells (MNC). A total of 1000 cells were scored directly on the slides from each patient for each sampling time (before and after X-ray exposure).

Data Analysis

All slides were analyzed by an experienced and blinded cytopathologist. The micronucleated cells (measure of DNA damage) were scored according to the criteria described by Sarto et al.²¹ For cytotoxicity, the following nuclear alterations were considered, as described by Tolbert et al.²²: pyknosis, karyolysis, and karyorrhexis (Figure 1a–d). A total of 1000 cells were assessed per person in this study for the micronucleus frequency and other parameters of cytotoxicity. The results were calculated by assessing % of altered cells only. Results are expressed in percentages. Similar analyses were established in previous published studies.^{11,14,16,19,20}

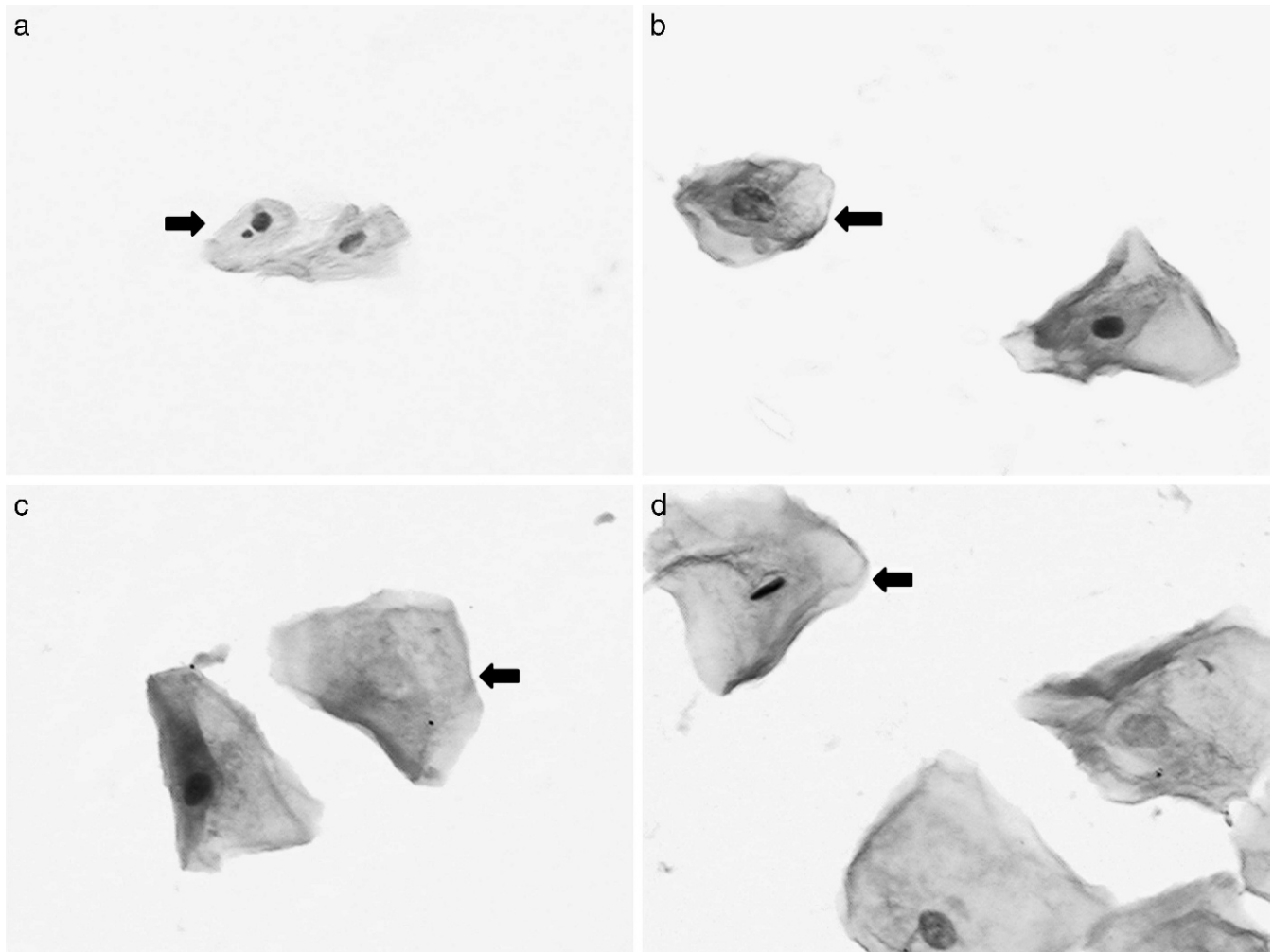


Figure 1. Nuclear alterations evaluation following cone beam computed tomography (CBCT) or radiograph exposure (400 \times magnification, Feulgen/Fast Green stain): (a) micronucleated (arrow) and normal cells, (b) karyorrhexis (arrow), (c) karyolysis (arrow), and (d) pyknosis (arrow).

Statistical Methods

The paired-samples *t*-test and the Wilcoxon test were used to compare the frequencies of nuclear alterations related to cytotoxicity and mutagenicity, respectively, before and after radiation exposure in the groups. To evaluate the pre- and postradiation differences in the frequencies of nuclear alterations related to cytotoxicity and mutagenicity between groups, the independent-samples *t*-test and the Mann-Whitney test were employed, respectively. The level of statistical significance was set at 5%.

The reliability of the evaluation was verified by pictures of the 600 BEC used in this study. These cells were numbered and classified according to their nuclear characteristics, as follows: normal, pyknosis, karyolysis, karyorrhexis, and MNC. After 30 days, these cells were reclassified, and the Kappa test was applied to investigate the concordance between the two evaluations.

RESULTS

According to the Kappa test, the concordance was adequate (Kappa value = 0.752). Table 1 shows the frequency of MNC (mutagenicity) and other nuclear alterations (cytotoxicity) in children undergoing radiographs or the CBCT necessary for orthodontic treatment. Before X-ray exposure, the mean frequency of MNC was 0.025% for the CBCT group and 0.008% for the Radiographic Set group. No statistically significant differences ($P > .05$) were noted after ionizing radiation exposure, showing no mutagenic effect. However, a significant increase in other nuclear alterations was observed after these exams, specifically, karyorrhexis, pyknosis, and karyolysis, evidencing cytotoxicity ($P \leq .001$ for the CBCT group and $P \leq .007$ for the Radiographic Set group). This increase was greater in the CBCT group ($P \leq .044$). These data are summarized in Table 1. None of the evaluated

Table 1. Frequency (%) of Micronucleated Cells (MNC) and Other Nuclear Alterations (Karyorrhexis, Pyknosis, and Karyolysis) in Children Undergoing Radiographs or Cone Beam Computed Tomography (CBCT) and Differences Between “After” and “Prior to” Exams Periods (Values Are Means \pm Standard Deviation [SD]; *, **, and *** Were Significant)

	Prior to Exam		After Exam		Difference After–Prior To	
	Other Alterations	MNC	Other Alterations	MNC	Other Alterations	MNC
CBCT (n = 24)	12.4 \pm 4.6	0.025 \pm 0.07	16.4 \pm 4.1*	0.033 \pm 0.08	4.0 \pm 4.0***	0.008 \pm 0.07
Radiographs ^a (n = 25)	12.2 \pm 5.3	0.008 \pm 0.03	14.4 \pm 5.1**	0.024 \pm 0.05	1.9 \pm 3.3	0.016 \pm 0.06

^a The radiograph data were taken from our previous study (<http://www.angle.org/doi/abs/10.2319/072311-468.1>).²⁰

* $P < .001$ vs children prior to exam exposure; ** $P < .007$ vs children prior to exam exposure; *** $P < .044$ vs children of radiography group.

children were exposed to other known genotoxic agents.

DISCUSSION

Buccal epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion.⁵ Add to that the knowledge that oral malignant neoplasms are the sixth most common neoplasm in the world,²³ and 90% of all oral human cancers originate from epithelial cells.²⁴ These facts highlight the advantage of the MN assay, an in vivo exam that elucidates the effects of toxic agents directly on a target tissue, the buccal epithelium. The limited cost, ease of counting, person-time required, and precision obtained from scoring large numbers of cells¹⁵ improve the popularity of this noninvasive method.

Damages that lead to the formation of micronuclei take place in the basal layer of the epithelial tissue, where cells undergo mitosis. The rapid turnover of epithelial tissues brings the cells to the surface, where they exfoliate.²⁵ In general, cells take 7–16 days to emerge to the surface and exfoliate.²⁶ For this reason, exfoliated oral mucosa cells were collected immediately before ionizing radiation exposure and after 10 days, in accordance with similar studies.^{3,11,13} This period allowed time for the basal layer that was exposed to radiation to mature and be collected when exfoliated.

Human biomonitoring studies in buccal cells involve several confounding factors, such as age, lifestyle, oral hygiene (eg, mouth rinse utilization), dental health, and smoking and alcohol use.⁵ These factors were controlled in our study. The sample comprised only children between 8 and 15 years of age with suitable oral hygiene and dental health. Children are minimally affected by confounders such as cigarette smoking, drinking habits, occupational exposure, and lifestyle (mainly dietary factors), which are factors of great concern in adults.²⁷ Moreover, each patient was considered to serve as his own control. Therefore, any effect of other genotoxic agents must have been present in the first cell count. Therefore, potential differences between the first and second counts can be attributed to radiation.¹² Some studies have pointed

toward a relationship between age and MN occurrence,^{13,27} whereas others have not.^{3,14} As a result of the homogeneity in casuistic, it was not possible to correlate the frequency of MNCs with age in this setting.

Mutagenicity can be effectively assessed by the MN assay.^{2,5} The MNC frequencies were not significantly different before and after X-ray exposure in our sample. These results contrast with those of other authors,^{2,3,28,29} who reported higher rates of chromosomal aberrations subsequent to X-ray exposure. However, despite the larger radiation dose in our investigation, many similar studies^{10–16} involving dental radiographs, PAN or LAT only, showed similar results (ie, no mutagenic characteristic was evidenced by the MN test). Similarly, recent studies with adult patients undergoing orthodontic radiographs¹⁹ and CBCT showed no mutagenic effects by the MN test.¹⁸ The large increase in MNC after the radiographs were taken was not statistically significant because the distribution of these cells was not homogeneous in the sample. This is evident by the high standard deviation in this group before and after the examination.

Differences in radiation dose, frequency of exposition, type of cells evaluated, and site of collected cells may influence the results of the MN test. Some authors investigated patients undergoing radiotherapy five to six times per week for 5–7 weeks,^{2,28} others observed effects of frequent occupational exposition to low doses of X-ray,²⁹ and still others pointed out results of only one dental radiographic exposure,^{3,10–17,19} and the literature shows that MN, MNC,³⁰ and cellular death¹² increase with radiation dose. With regard to the different cells employed in the MN assay, radiotherapy is a potent clastogenic agent in circulating lymphocytes and BEC of head-and-neck cancer patients.² However, lymphocytes are more sensitive than BEC when detecting chromosomal aberrations caused by anti-cancer drugs.^{9,26} Additionally, in the cytogenetic studies of dental radiograph effects, different sites were selected for collection of buccal epithelial cells: buccal cheek mucosa,^{10,11,13,15–17} lateral border of the tongue,^{12,16} and keratinized mucosa of the upper dental arch.³ One study¹⁶ showed that the lateral border of the tongue is a more sensitive site with regard to cytotoxic insult induced by ionizing radiation combined with continuous cigarette smoke exposure when compared

with the cheek buccal mucosa. The unique research that revealed the genetic damage capacity of PAN was contained in the unique study³ that utilized keratinized mucosa of the upper dental arch. These facts emphasize the need for more comparisons between different buccal sites, and they help us to explain the divergence found in the studies of radiation biological effects. Based on our findings, we assume that there are no mutagenic effects related to radiographs or CBCT utilized for orthodontic planning in children.

Researchers^{10,22} have called attention to nuclear changes other than MN that characterize cellular death and may increase the sensitivity of tests to detect genotoxicity. Thus, cytotoxic effects were investigated through the frequencies of karyorrhexis, karyolysis, and pyknosis. Contrary to genetic damage, cellular death was induced by radiographs and CBCT, as indicated by the statistically significant differences ($P \leq .001$ for the CBCT group and $P \leq .007$ for the Radiographic Set group) between values before and after X-ray exposure, in agreement with the findings of other studies.^{10,11,14,19} These results reinforce the idea that X-rays are cytotoxic, and based on the knowledge that cytotoxicity interferes with micronucleus induction because some MNC are inevitably lost after a cytotoxic insult,¹¹ the lack of mutagenic effects of X-ray can be accepted.

Nevertheless, repeated exposure to cytotoxic agents can result in chronic cell injury, compensatory cell proliferation, hyperplasia, and, ultimately, tumor development. These cytotoxic/nongenotoxic agents act by interfering with molecules intimately involved in cell growth and cell death. Increased cell proliferation appears to be a unifying feature of epigenetic carcinogens. Proliferation may increase the risk of mutation within target cells and may also be important in selective clonal expansion of initiated cells.³¹

Comparisons show that the CBCT group presented a larger increase in cell death than the Radiographic Set group. This might have happened once the CBCT group received a greater radiation dose due to the protocols used in orthodontic treatment planning for this research, which were in accordance with the recent radiation doses described in the literature. The total radiation doses in the Radiographic Set group varies between 195 and 205 μSv , and in the CBCT group (FOV22 + FOV13) they varied between 287 and 304 μSv .³²⁻³⁴ The fact that cytotoxicity is closely related to the amount of radiation received was shown in patients exposed to a repetition of a PAN (14–24 μSv).¹²

CONCLUSIONS

- CBCT or radiographs that are requested for orthodontic planning can induce cytotoxic effects in oral mucosa cells.

- The CBCT group experienced more cell death, and that might have happened once this group received a greater radiation dose due to the protocols used in orthodontic planning for this study.
- Considering that cellular death and nongenotoxic mechanisms of carcinogenesis are closely related, CBCT or radiographs should be used when essential to treatment planning following the ALARA (As Low As Reasonably Achievable) principle, because cellular death was induced.

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